A CONTRIBUTION TO A STUDY OF THE PHYSIOLOGY OF DECAY IN APPLES.

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(Ten Text-figures.)

[Read 28th November, 1945.]

Introduction.

Fungal decay and physiological breakdowns are responsible for the wastage which occurs in apples during storage and it is the object of this paper to discuss briefly some of the factors relating to fungal decay.

The moulds most frequently responsible for damage in apple storage are *Penicillium expansum* and *Gloeosporium album*.

P. expansum is a typical wound parasite and most frequently gains entrance through mechanical injuries such as stem punctures, insect injuries and necrotic tissue. The lesions are soft and watery, light brown in colour, and are not restricted to any one position but may occur on the stem, cheek, or calyx ends, or may develop as core rots arising from spores that have penetrated the open calyx canal. About 80% of the storage decay has been attributed to this blue mould soft rot. The fact that *P. expansum*, given a favourable medium, can make a much better start at 0°C. than any other fungus helps to explain its common occurrence in cold storage (Brooks and Cooley, 1917).

Gloeosporium album produces what is known as the 'bull's eye rot'. The rot develops as a flat or concave, fairly firm, lesion, chestnut-brown in colour. The lesion has a typical zoned appearance with a well-defined margin and the flesh beneath the rot is dry and leathery. Like the blue mould, lesions produced by *G. album* may occur in any position on the apple. Acervuli situated concentrically around the point of infection appear when lesions are mature. This rot develops more slowly than the blue mould soft rot and is prevalent in the later part of the storage life.

Experiments on these moulds carried out by the writer and discussed in this paper are set out in two sections.

Under Section 1 the following factors were examined for the effect they produced on the rate of decay in apples under storage conditions: variety of apple, maturity at picking, district and locality of orchard.

Under Section 2 a study was made of the effect of the following factors on the growth rate of *P. expansum* and *G. album*: (*a*) hydrogen ion concentration; (*b*) temperature; and (*c*) age of spore.

Section 1.

Material and methods.

The investigations on fruit were carried out at the Food Preservation Laboratory of the Council for Scientific and Industrial Research, Homebush, where storage rooms with properly controlled temperatures were made available for the storage of the fruit during the years 1941 and 1942. All fruit on arrival at the Food Preservation Laboratory was stored immediately at 34°F. until removal for inoculation.

In 1941, the fruit was obtained from Batlow and Orange. The varieties used were Delicious and Granny Smith and there were three periods of picking: first, second, and third; the first and third picking refer to early and late maturity.

All inoculation procedure was standardized and although inoculations took place over a period of time, spores of similar age were used in all inoculations. The spores

of *P. expansum* were taken from cultures which had been incubated at 20°C. for 10 days; *G. album* from cultures incubated at 20°C. for 21 days. The method of inoculation was a modification of that developed by Granger and Horne (1924). A sterilized cork-borer was inserted obliquely into the apple to a specified depth, then withdrawn lifting up a triangular flap of tissue for the insertion of the inoculum (250 spores per loop). The flap was then closed and sealed with sterile paraffin. The fruit was then wrapped and packed in cases and stored at 32°F. and 40°F.

The apples were sampled five at a time and the rate of decay was measured by Gregory and Horne's (1928) method of radial advance.

During the following year (1942) a more detailed examination of fruit from one district was carried out using the same technique. For this investigation early and late maturity fruit were obtained from the following orchards at Orange: (*a*) Delicious fruit from the orchards of Messrs. Fox-Martin, Scott, Tonking and Coote; (*b*) Granny Smith fruit from Scott's orchard.

The inoculated fruit was stored at 40° F. in 1942.

EXPERIMENTAL RESULTS.

There was a general tendency for the fruit at the late maturity stage to be more susceptible to invasion by both fungi. The effect was more striking in fruit from Orange, and where maturity differences were pronounced, the effect was observed at 32°F. and 40°F. An example of the effect of late maturity picking on the rate of rot development is illustrated in Fig. 1.

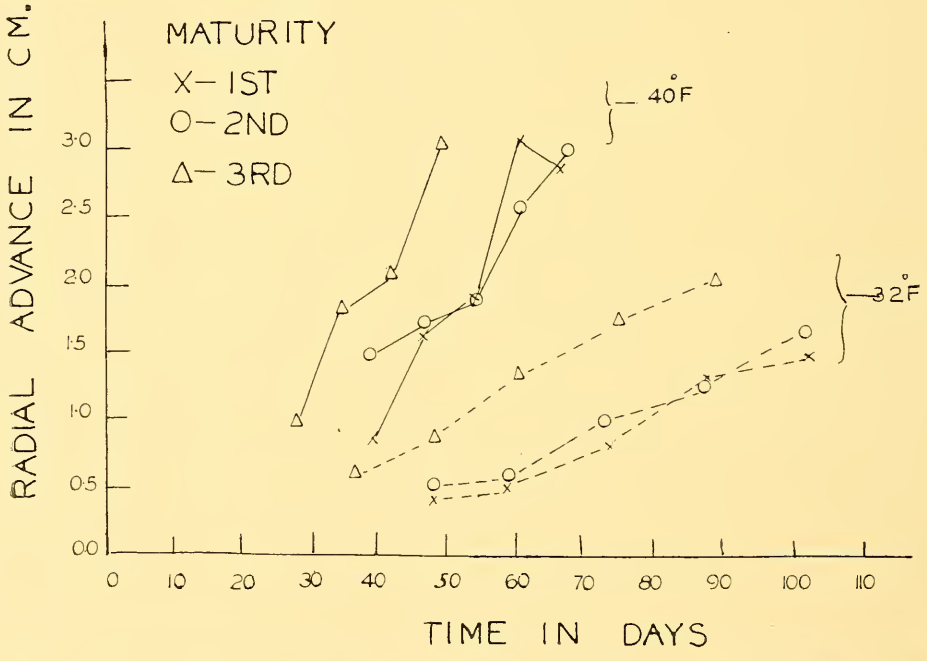

Fig. 1.—The effect of maturity on the radial advance of *P. expansum* in Delicious apples from Orange.

Delicious fruit rotted at a faster rate than the Granny Smith fruit. This varietal effect was most pronounced in the fruit from Batlow inoculated with *G. album* stored at 40°F. (Fig. 2).

It was observed that, in fruit from Batlow, greater differences occurred in the rate of decay between varieties than in the same variety at different maturities. In fruit from Orange the differences due to maturity were more pronounced than the varietal effect.

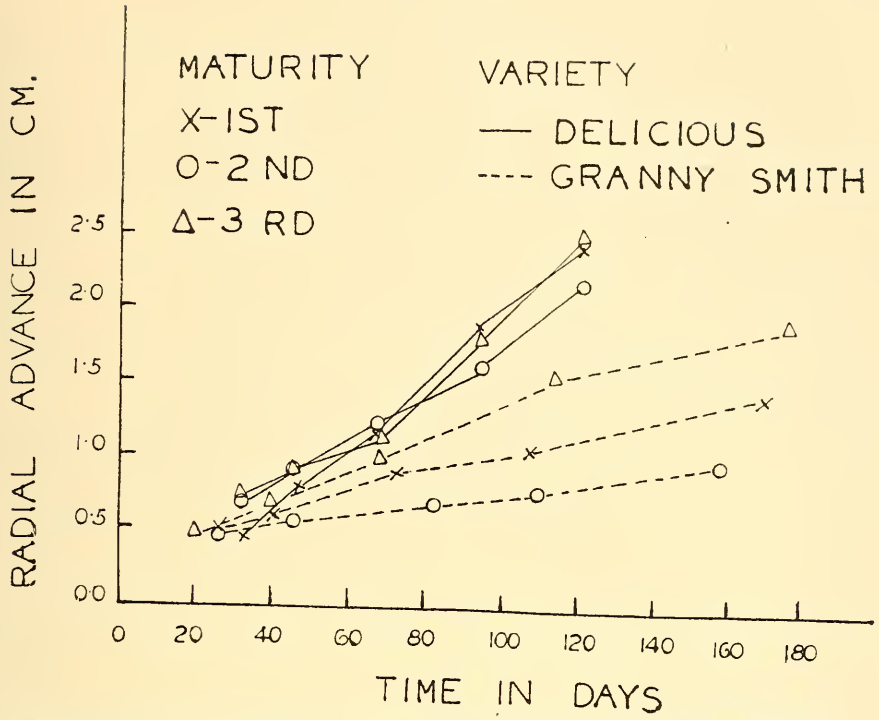

Fig. 2.—The effect of variety on the radial advance of *G. album* at 40°F.—Batlow.

A district-effect was observed in the Delicious fruit: fruit from Batlow showed a marked tendency to decay at a faster rate than fruit from Orange (Fig. 3). No such district-effect was shown by the Granny Smith fruit.

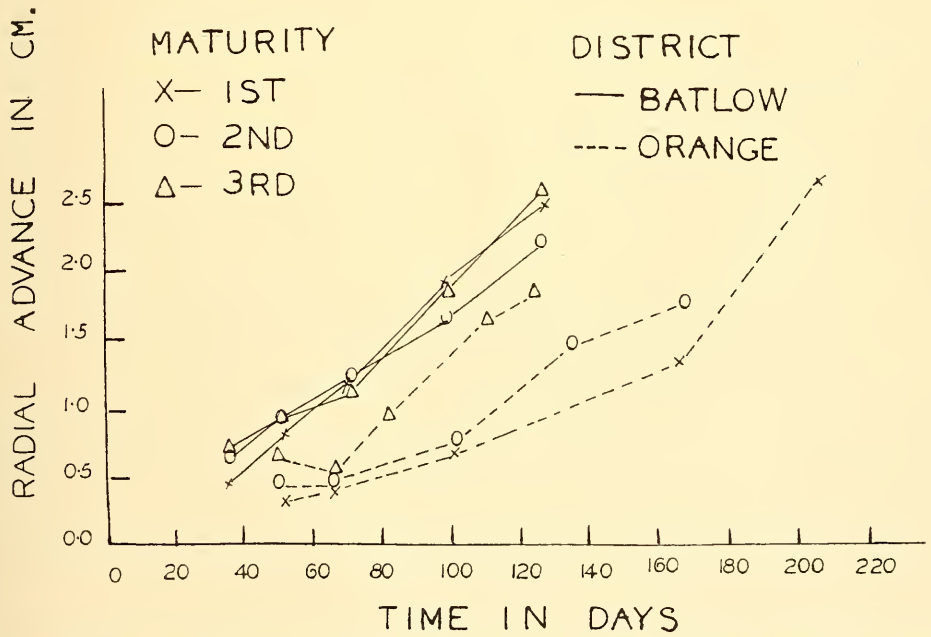

Fig. 3.—The effect of district on the radial advance of *G. album* at 40°F.—variety, Delicious.

Data obtained from the investigation carried out in 1942 indicated that maturity differences were not as marked as varietal differences. There was, however, the same general tendency for the late maturity fruit to be more susceptible to fungal invasion than the early maturity.

Locality of orchard had no effect on the growth of *P. expansum*. Early maturity fruit from Scott's orchard, inoculated with *G. album*, rotted at a slower rate than fruit from the other orchards. Differential rotting was not apparent in the late maturity fruit (Fig. 4).

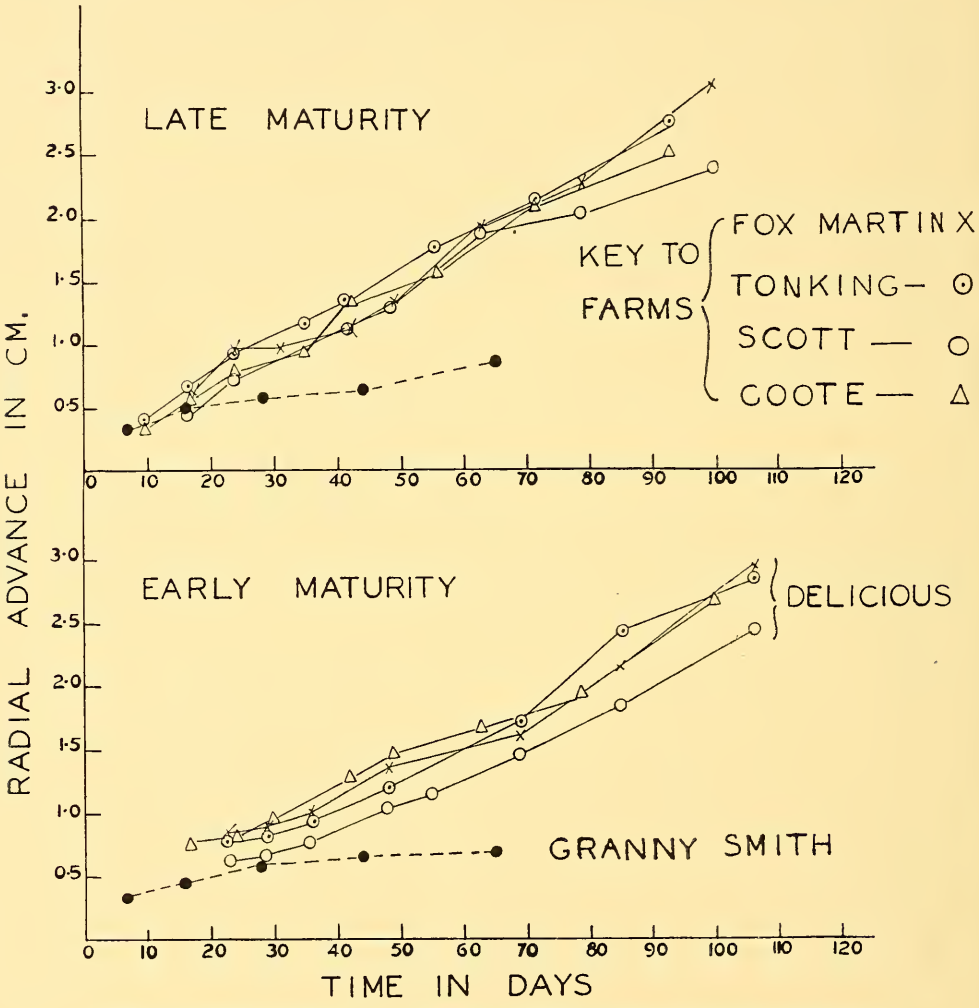

Fig. 4.—Radial advance of *G. album* in Granny Smith and Delicious apples.

With a view to determining the cause of this differential rotting between varieties and maturities, the following possibilities were investigated: (*a*) hardness of tissue; and (*b*) pH of sap.

To determine whether hardness was correlated with susceptibility to decay, an examination was carried out on the hardness of the apples. This was measured by the resistance of the cells to a standard plunger. No significant difference between varieties and maturities was seen in the hardness of the fruit.

The pH of the expressed juice of apples of all maturities and varieties was determined. Differences in hydrogen ion concentration between maturities were not

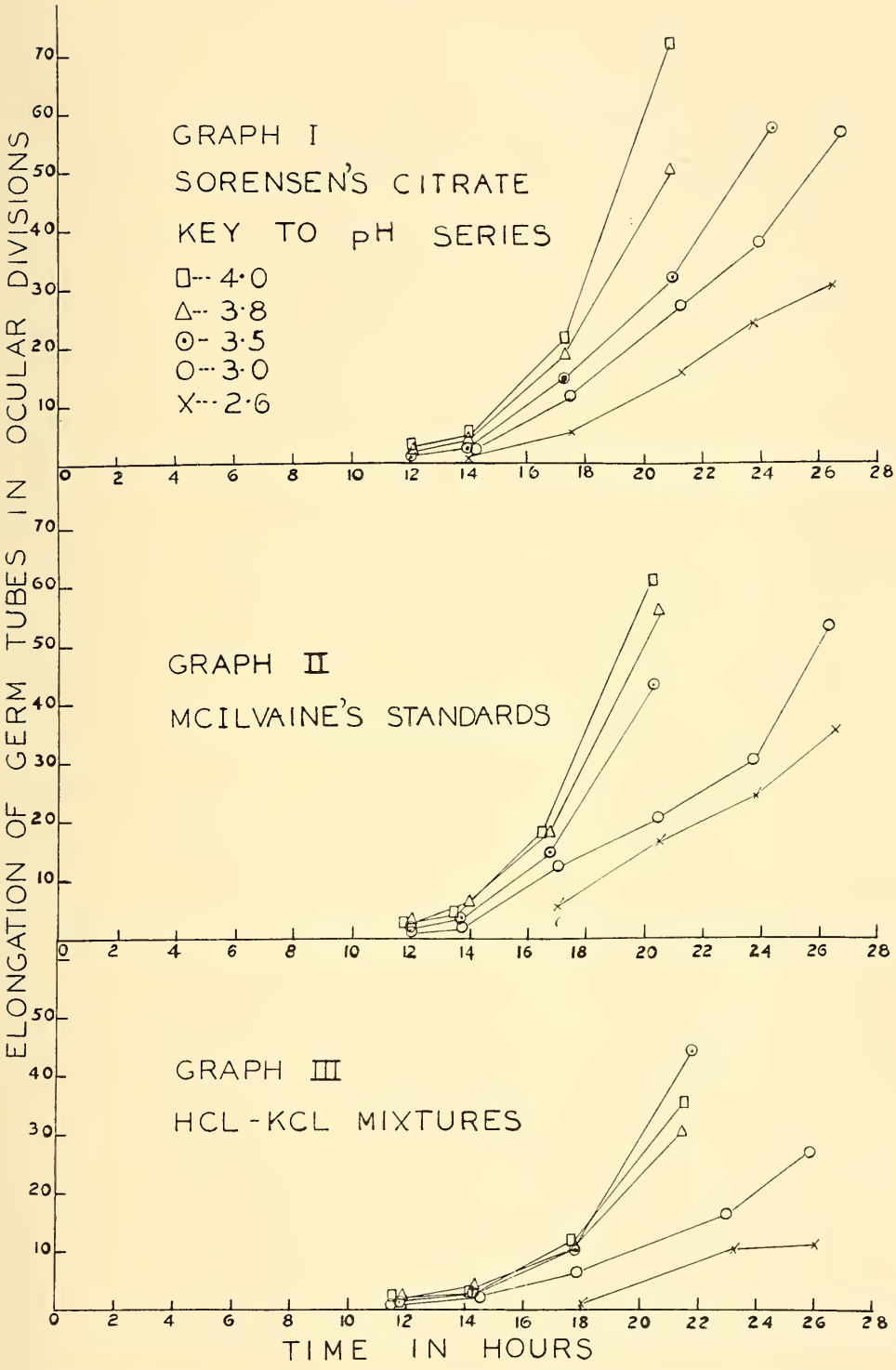

Fig. 5.—Elongation of germ tubes of *P. expansum* in relation to hydrogen ion concentration.

significant but the hydrogen ion concentration of Granny Smith juice (pH 3·6) was more acid than that of Delicious juice (pH 4·0). Successful attack is due partly to chemical changes in the cell such as hydrogen ion concentration, sugar content and pectin. It seems possible therefore that this difference in hydrogen ion concentration observed between varieties may account for the differential radial advance of the rots. This aspect was therefore made the subject of further experiment (Section 2).

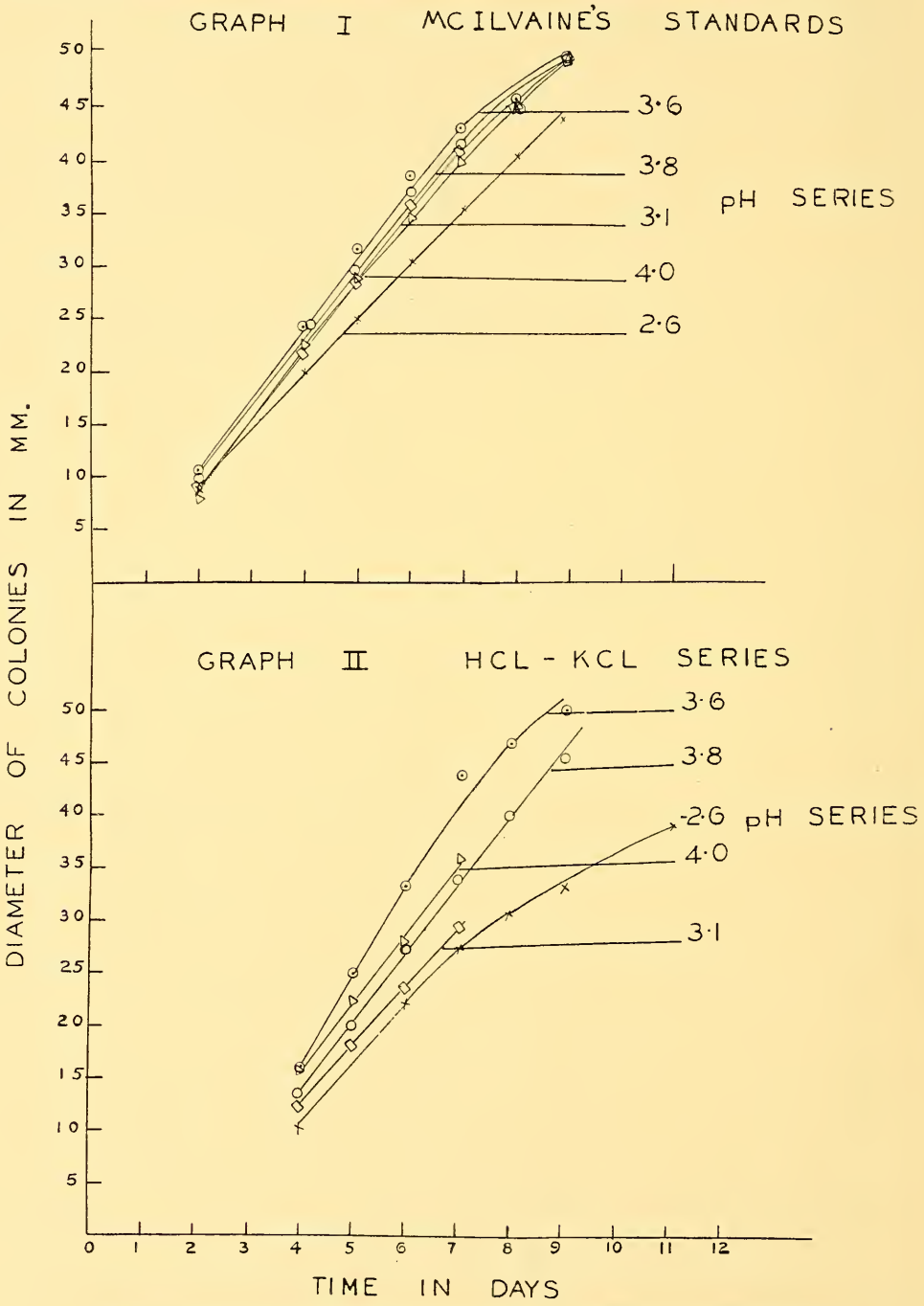

Fig. 6.—The growth of *P. expansum* in relation to hydrogen ion concentration.

SECTION 2.

(a) The Effect of Hydrogen Ion Concentration on Growth.

Studies of the moulds in culture comprised both germination and growth experiments.

To determine whether hydrogen ion concentration accounted for differences in the rate of decay between varieties, the fungi were grown at pH values closely approximating to the hydrogen ion concentration of the apple juices. The moulds were grown in potato-dextrose broth at the following pH range: 2·6, 3·1, 3·6, 3·8, 4·0 and buffered by McIlvaine's Standards, or Sorensen's Citrate, or HCL-KCL Mixtures.

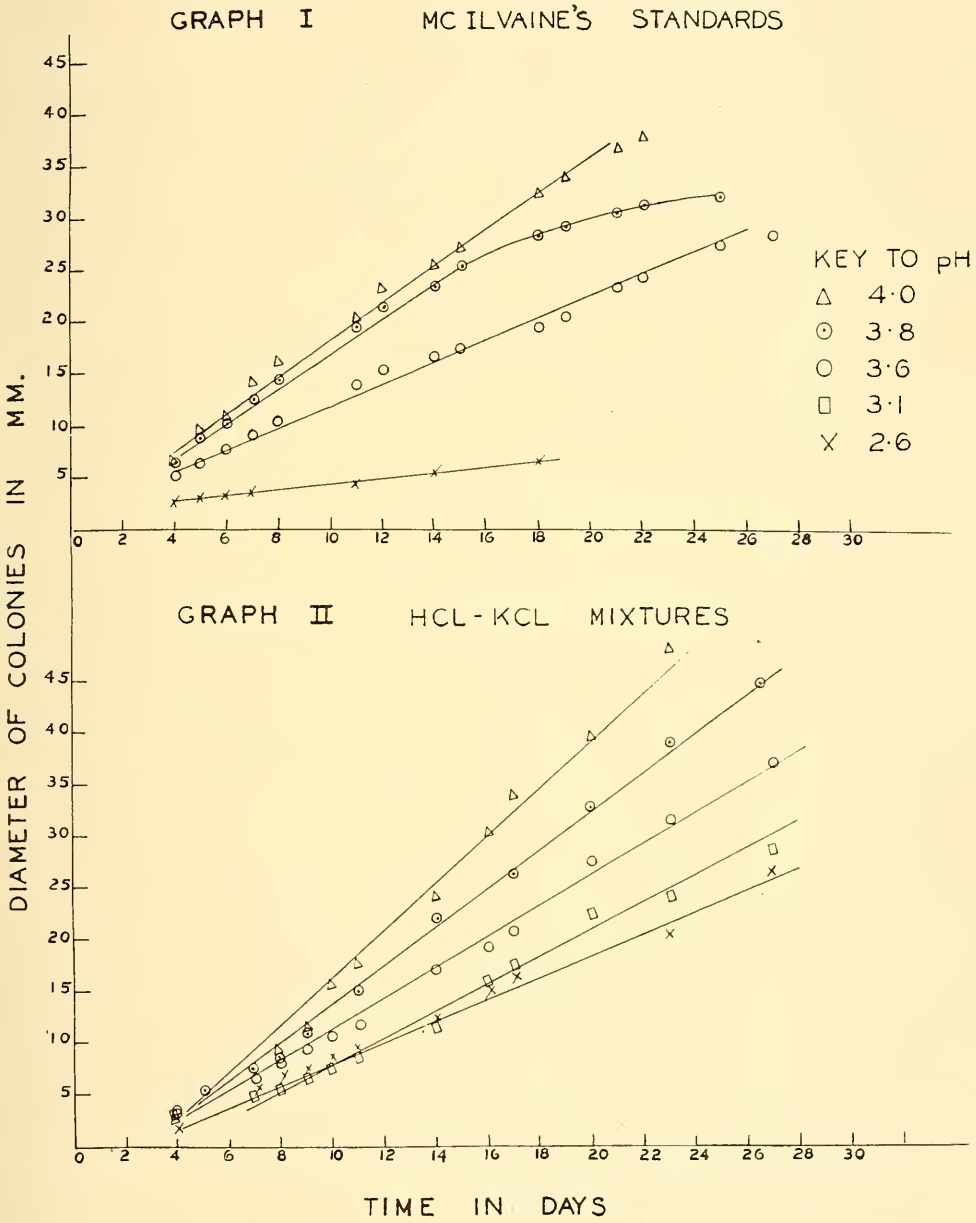

Fig. 7.—The growth of *G. album* in relation to hydrogen ion concentration.

Sterile plaster of Paris discs were placed in Petri dishes containing 20 c.c. of buffered broth. The growth rate was measured by recording the diameter of the colonies and the discs were marked with pencil in mm. to facilitate readings (Cash, 1942). Each disc was inoculated in the centre with a 1 mm. mycelial square and the experiment was carried out in replicates of five at each pH value. To eliminate the effect of staling on growth, the discs were transferred daily under aseptic conditions to Petri dishes containing fresh culture solution of the same pH.

Results.—The examination of spore germination of *P. expansum* showed that pH did not affect the latent period (Fig. 5, Graphs I and II). Irregular sprouting of the germ tubes and an increase in the latent period at pH 2·6 in the HCL-KCL buffer series suggest that unfavourable conditions were due to the type of buffer rather than to unfavourable pH (Fig. 5, Graph III). Germination studies on *G. album* were not carried out.

The growth of *P. expansum* in McIlvaine's Standards series was independent of pH but a well-defined optimum was observed at pH 3·6 in the HCL-KCL buffer series (Fig. 6). *G. album* showed a maximum growth at pH 4·0 in all buffer types with a consistent decline on the acid side (Fig. 7).

Normal growth took place at pH 2·6 in the HCL-KCL buffer series but the cultures did not grow in McIlvaine's Standards series at a similar pH. This indicates the necessity for using more than one buffer type to determine whether the effect is chemical or due to hydrogen ion concentration.

(*b*) *The Effect of Temperature on Growth.*

The technique used for observing the latent period, percentage germination and elongation of the germ tubes was to spread a dilute spore suspension over agar films

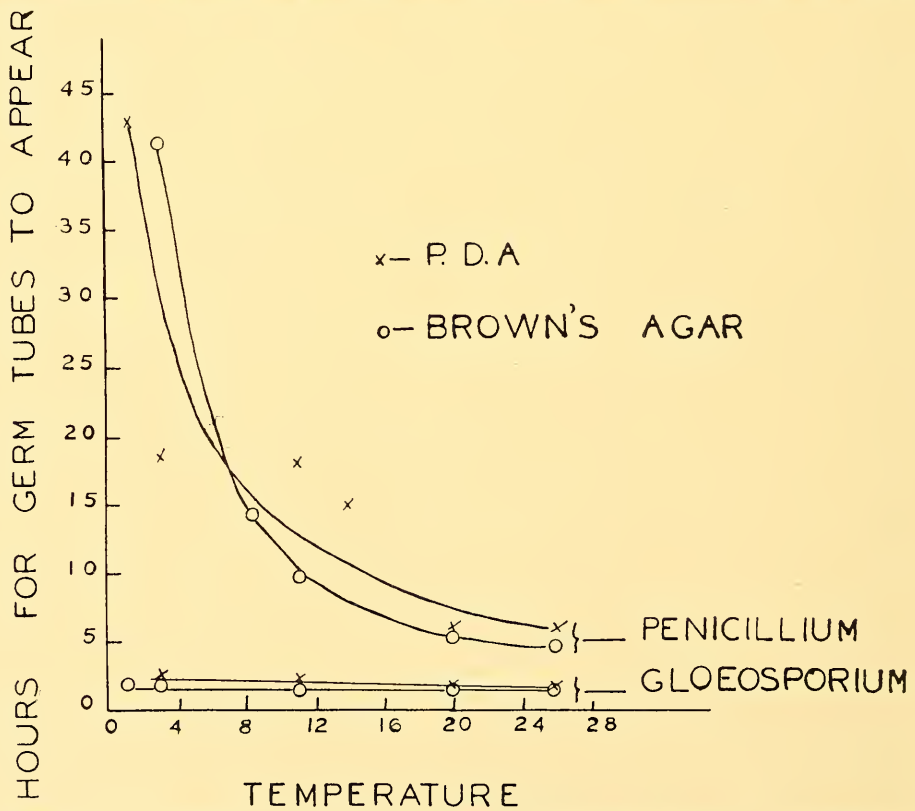

Fig. 8.—The effect of temperature and media on the latent period of *P. expansum* and *G. album*.

corresponding to the type of media to be used in the growth studies. For the examination of the effect of temperature on growth, colonies were grown in Petri dishes containing 18 c.c. of 2% potato-dextrose agar, malt agar or Brown's agar at 1·3°C., 3°C., 11°C., 14°C., 20°C. and 26°C. The experiment was carried out in replicates of five at each temperature and growth was measured by recording the diameters of the colonies.

Results.—The results given in Fig. 8 show that the temperature has no effect on the latent period of *G. album*. As the temperature decreases there is an increase in the latent period of *P. expansum*. In all cases 100% germination was obtained. Fig. 9

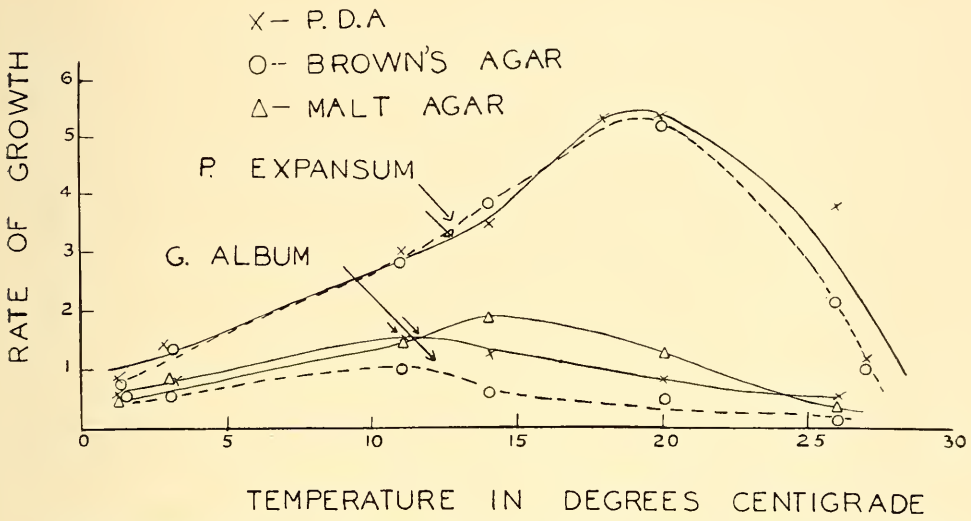

Fig. 9.—Growth rates of *P. expansum* and *G. album* on different media and at different temperatures.

shows that, within the range of the experiment, optimal conditions for the growth of *P. expansum* were at 20°C., whereas the optimal growth rates of *G. album* varied slightly with the type of the medium and were dependent on time.

(*c*) *The Effect of Age of Spore on Germination at Different Temperatures.*

Germination studies were carried out with the spores of *P. expansum* and *G. album* of different ages to determine whether the age of the spore lying on the fruit could affect the initial rate of penetration into the fruit. Spores were obtained from cultures of *P. expansum* of the following ages: 5, 42, 104 and 217 days, and from *G. album* 64, 132, 179 and 212 days old. All cultures used to determine the effect of age of spore on germination had been stored at 3° C. Germination studies were carried out at 1·3°C., 14°C. and 25°C.

Results.—Within the experimental range, spore age did not affect the latent period of either *P. expansum* or *G. album*. Germination was 100%. Optimal rates of germ tube elongation of *P. expansum* were observed in the youngest spores with a progressive decline as the spores age (Fig. 10). The spores of *G. album* showed a maturation effect, the optimum rate for the elongation of the germ tubes varying with the temperature.

This examination on the germination of spores of different ages may possibly suggest that spore age does not affect initial penetration into the fruit but may play a part in the rate of rot development. It is possible that the different rates observed in the elongation of the germ tubes may be an initial effect only and requires further investigation.

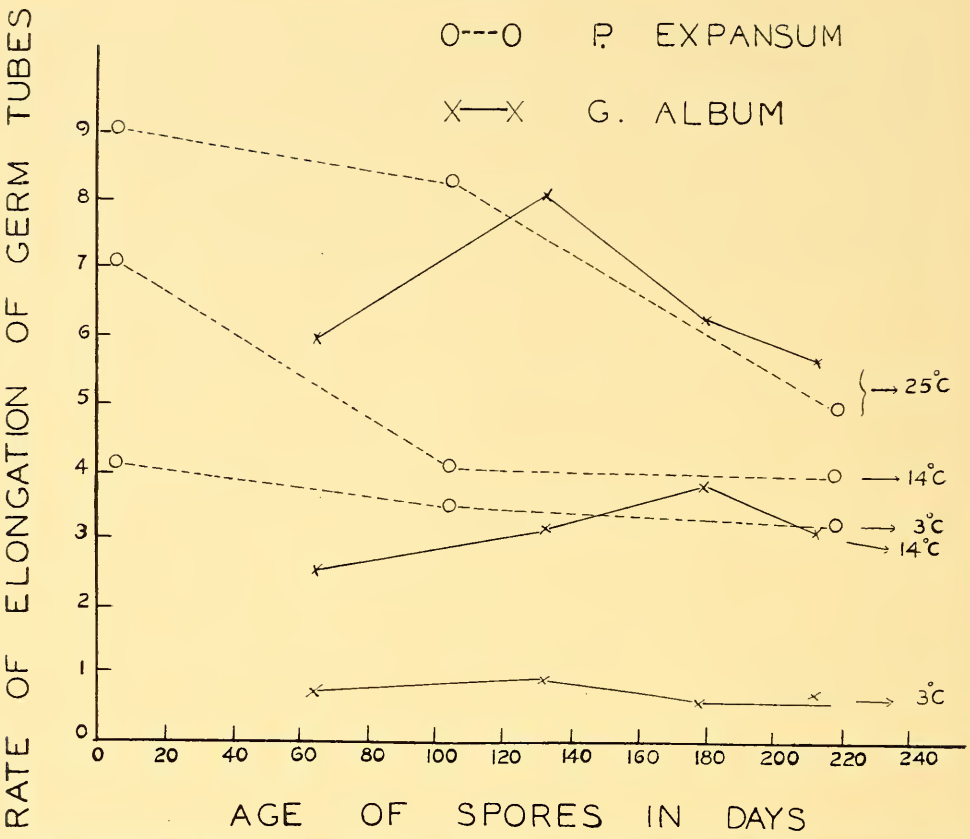

Fig. 10.—Effect of spore age on the rate of elongation of germ tubes of *P. expansum* and *G. album* at different temperatures.

Discussion.

Data obtained from these investigations present no adequate explanation for the differential decay observed between varieties. Results of experiments on the growth of *P. expansum* in culture at different hydrogen ion concentrations were not consistent with the data obtained on the radial advance in the fruit. Optimal growth rates for this fungus were observed at pH 3·6 in the HCL-KCL buffer series. This pH value closely approximates to the pH of the Granny Smith juice (pH 3·5), yet the rate of decay was faster in the Delicious fruit. At a hydrogen ion concentration similar to that of the Delicious fruit, *P. expansum* showed a decrease in the growth rate in culture. It appears, therefore, that other factors are responsible for differences in the rotting of fruit by *P. expansum*.

The differential rates of radial advance in apples by *G. album* showed a close correlation with growth in culture at different hydrogen ion concentrations, and, therefore, hydrogen ion concentration may also possibly offer an explanation of the differential rate of decay observed between varieties. The pH values found in apples of the same maturity showed so much variation that it was impossible to attribute differences in radial advance between maturities of the same variety to differences in hydrogen ion concentration.

It has been found by other investigators (Wilkinson, 1938) that resistance to invasion is influenced by manurial treatment. Wilkinson found that nitrogen manuring had a marked effect on the incidence of bull's eye rot, low nitrogen manuring showed a 10% wastage due to *G. album* and a high nitrogen manuring a 31% wastage. Though no data were obtained on the manurial treatments used on the orchards at Orange it was found that, irrespective of maturity, the locality of the orchards had

no effect on the rate of decay due to blue mould. Fruit from Scott's farm inoculated with *G. album* rotted at a lower rate than fruit from the other orchards, but it is doubtful whether this would be a manurial effect since this effect was not observed in fruit at the late maturity. However, for both types of rots, the varietal effect was more pronounced than any differences in orchard locality which may have been due to manuring. The chemical composition of the fruit may influence the rate of decay, but so far no chemical analyses have been carried out in conjunction with this investigation.

Since spore age has no effect on the latent period and percentage germination, it may be assumed that spores of these moulds of any age, on the surface of the fruit at the time of storage, are all capable of infection.

Summary.

The effect of some environmental factors on the rate of decay of Delicious and Granny Smith apples in storage has been studied and the following conclusions are presented:

1. It seems that district from which the fruit comes and variety of fruit have a marked effect on the rate of radial advance of the rot. Fruit at late maturity showed a general tendency to rot at a faster rate than fruit at early maturity.

2. Several suggestions have been put forward which may help to account for the differential decay observed between varieties but no completely satisfactory explanation can be offered at present.

3. Inherent qualities of the apples such as hardness of the tissue and skin colour have no measureable effect on the rate of decay.

4. Insufficient data prevent interpretation of the orchard locality and district effects but it is possible that nutritive conditions may influence rate of decay.

5. Although the studies in the physiology of *P. expansum* and *G. album* are incomplete the data obtained indicate that germination of the spores of *G. album* is not affected by temperature, but with a decrease in temperature, an increase in the latent period of *P. expansum* was observed.

6. Within the range tested, optimal conditions for the growth of *P. expansum* were observed at 20°C. The position of the optimum of *G. album* was found to be dependent on time.

7. Hydrogen ion concentration has a well-defined effect on the growth of *G. album*. Within the experimental range a maximum was found at pH 4·0, and there was a progressive decline in the growth rate as the culture media became more acid. The growth of *P. expansum* in broth buffered by McIlvaine's Standards was found to be independent of pH but an optimal effect was observed in the HCL-KCL series at pH 3·6. This indicates the importance of using more than one buffer type.

8. Age of spore was found to have no effect on the latent period and percentage germination of *P. expansum* and *G. album* at different temperatures.

Acknowledgements.

The writer wishes to express her thanks to Professor Eric Ashby in whose department this work was carried out, to Dr. J. R. Vickery for permission to use the facilities of the Division of Food Preservation, Council for Scientific and Industrial Research, Homebush, and to Dr. John McLuckie and Dr. Lilian Fraser for their helpful criticism in the preparation of the manuscript.

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